Karyotyping of Chromosomes in Human Bronchial Epithelial Cells Transformed by High Energy Fe Ions

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Introduction

Lung cancer induced from exposures to space radiation is one of the most significant health risks for long-term space travels. Evidences show that low- and high- Linear energy transfer (LET)-induced transformation of normal human bronchial epithelial cells (HBEC) that are immortalized through the expression of Cdk4 and hTERT. The cells were exposed to gamma rays and high-energy Fe ions for the selection of transformed clones. Transformed HBEC are identified and analyzed chromosome aberrations (i.e. genomic instability) using the multi-color fluorescent *in situ* hybridization (mFISH), as well as the multi-banding *in situ* hybridization (mBAND) techniques. Our results show chromosomal translocations between different chromosomes and several of the breaks occurred in the q-arm of chromosome 3. We also identified copy number variations between the transformed and the parental HBEC regardless of the exposure conditions. We observed chromosomal aberrations in the low- and high-LET radiation-induced transformed clones and they are imperfectly different from clones obtain in spontaneous soft agar growth.

Materials and Methods

- The cells were split at 80% confluence and cultured in T-75 flasks. They were fed twice a week, subculture once a week, and the cell were allowed to reach 80% confluence before the chromosomes were prepared.
- Chromosomes were prepared by dropping fixed metaphase cells onto clean wet slides. In situ hybridization of the chromosomes are performed using the mFISH and mBAND kit (MetaSystems, Altlussheim, Germany), according to the protocol recommended by the manufacturer.
- mFISH analysis was performed with all of the chromosomes painted in multiple color bands *and* mBAND analysis was also performed on clones that harbor damages on chromosome 3.

Data analysis:
Data processing and visualization-expression values were extracted using the Metsystem software (MetaSystems, Altlussheim, Germany).

Results

Figure 1 shows parental control chromosomes in which translocation between chromosomes 5 and 16 results in fusion of the two chromosomes, and also observed is chromosome 20 trisomy.

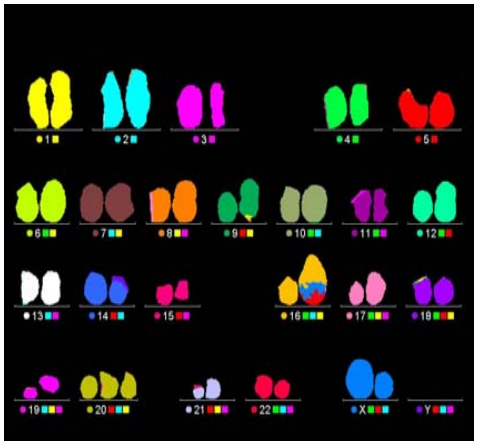


Figure 2a and 2b are karyotypes of unexposed single clone culture in soft agar. The cells from the single clone showed diversity of karyotypes when analyzed. In both figures, a new translocation involving chromosome 3 and the X chromosome result in fusion. Other aberrations are observed and they are: fig. 2a shows increase in chromosomal numbers 94 and chromosomal breakage of chromosome 7, including extra chromosome 8. Fig. 2b also shows increase in chromosomal numbers 84 and missing chromosomes 5, 7, 11, 19, 20, 22, and X.

Figure 2a

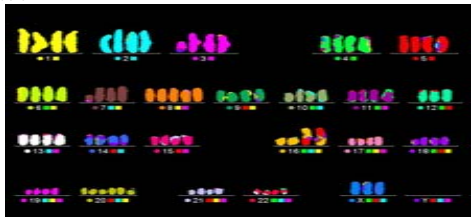


Figure 2b

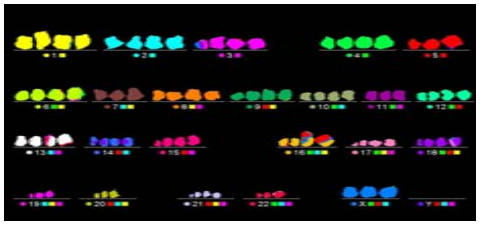


Figure 3 is an mBAND analysis breakpoint of chromosomes involved in translocation near the centromere. Another breakpoint is near the telomere of the long arm.

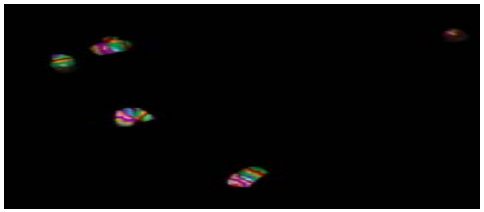
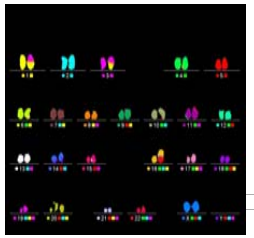
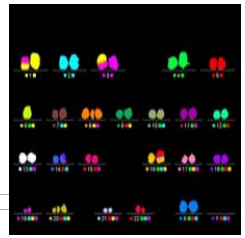


Figure 4a and **Figure 4b** show 3kt-ff-2a (p0) and 3kt-ff-2a (48) irradiated with Fe ion of 1Gy shows translocation between chromosome 1 and 3.



4a



4b

Figure 5 shows 3kt-ff-2a (48) shows chromosome 3 with breakage on q-arm.

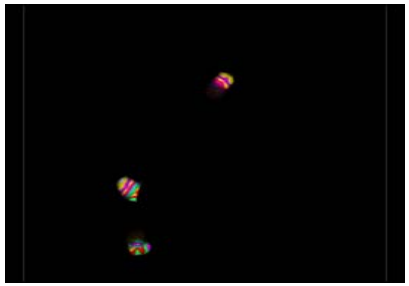
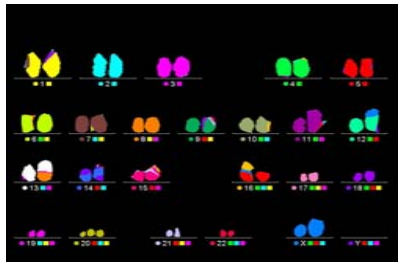


Figure 6 shows irradiated R53-r2g with gamma ray 2GY; and translocation results in fusion of chromosome 8 and 13 and chromosome 12 and the X-chromosome.



Conclusions

- The transformed cells have increased in chromosomal numbers greater or less than 46. Doubling of chromosome numbers is a signature of tumor.
- Chromosomal aberration was observed in non-irradiated HBEC-3kt cultured in soft agar, which is an indication of chromosome instability that leads to tumor development.
- It is most likely that the untreated transformed single clone cell undergoes unequal segregation of chromosomes in two daughter cells that results in an increasing number of chromosomes during mitosis, particularly in the anaphase stage.
- Our results for translocation and fusion of chromosomes are pronounced with chromosome 3 and the X-chromosome, which is detected and confirmed by mBAND pattern.

References:

1. Seongmi Park, Michael Peyton, Luc Girard, Yang Xie, John D Minna, and Michael D Story, Distinct transcriptome profiles identified in normal human bronchial epithelial cells after exposure to γ-rays and different elemental particles of high Z and energy.
2. Bioanalytical Chttp://www.umanitoba.ca/institutes/manitoba_institute_cell_biology/MICB/www.itmhrt.ca/students/files/pdf_11.pdfore.